

THE RELATION OF MORTALITY AFTER ENDOMIXIS TO
THE PRIOR INTERENDOMICTIC INTERVAL IN
*PARAMECIUM AURELIA*¹

BERNICE FRANCES PIERSON

(From the Department of Zoölogy, The Johns Hopkins University)

Investigators of endomixis in *Paramecium* have frequently observed that animals often die during or soon after endomixis. According to Erdmann and Woodruff (1916), few *Paramecium caudatum* survived endomixis. In *Paramecium aurelia*, Caldwell (1933) found that death occurred 2.85 to 9.40 times more frequently at endomixis than in the middle of the period between endomixes. The present paper is a presentation of the results of a study of one of the factors determining such deaths, namely, the length of the preceding interendomictic interval. As will appear below, when the prior interendomictic interval is unusually long, endomixis results in a greater percentage of deaths than when the interval is of the ordinary duration. Moreover, the greater the interendomictic interval, the greater is the mortality resulting from endomixis, until, after very long intervals, endomixis invariably results in death.

In order to investigate this question, it was essential to have available, simultaneously, lines with normal interendomictic intervals and sister lines with unusually long intervals. This was accomplished by employing recently developed methods of inducing endomixis (Sonneborn, 1937) and of obtaining lines with long interendomictic intervals (Sonneborn, 1938). To induce endomixis, the surplus animals from daily isolation lines of cultivation were collected in a small amount of fresh culture medium and kept at 31° C. for a few days until endomixis occurred. To obtain lines with long interendomictic intervals, daily isolation lines which went into endomixis were replaced by sister lines which had not yet gone into endomixis. In this way, lines with long intervals are selected for study while the lines with shorter intervals are eliminated.

Using these techniques, the following experiment was performed. The vegetative descendants of a single endomictic individual were cultivated as 24 daily isolation lines of descent for 165 days. During this time, all lines that went into endomixis were eliminated and re-

¹ This work was suggested by Dr. T. M. Sonneborn, to whom I wish to express my sincere appreciation for his helpful advice and assistance.

placed by surplus animals from sister lines; so that at all times this group (I, Fig. 1) consisted of 24 lines that had not been in endomixis since the start of the experiment. At five successive intervals of 21 to 31 days, surplus animals from this group were induced to go into

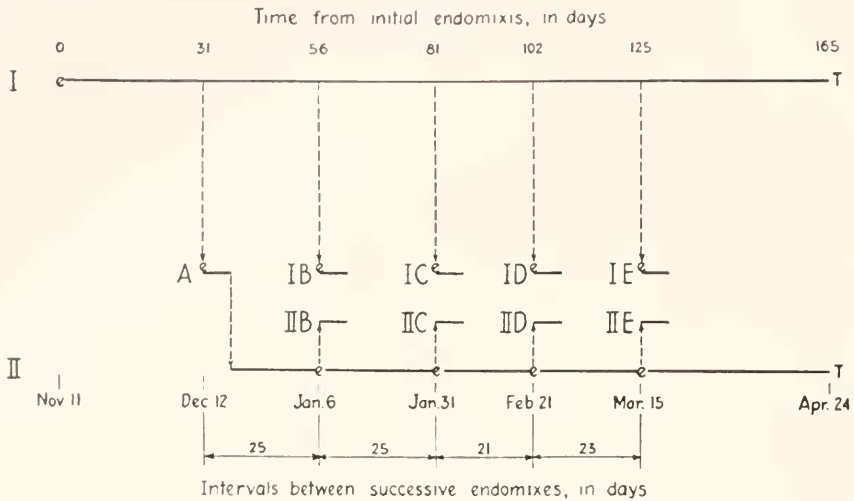


FIG. 1. Plan of Experiment

The horizontal lines (solid) represent groups of isolation culture lines. Long, solid line "I" represents a group of 24 isolation lines from which all endomixities were eliminated. Long, solid line "II" represents a group of 24 isolation lines, interrupted periodically (at points marked *e*) for the induction of endomixis in small mass cultures. The short, solid, horizontal lines represent groups of isolation lines carried for 15 generations. The length of the short lines corresponds to the average number of days that the 15 generations lived in all the groups.

The vertical lines (broken) connect the source groups (represented by long, solid, horizontal lines) with their derived groups (represented by short, solid, horizontal lines). In each case, the derived group began with animals in endomixis taken from mass cultures of animals from the source group.

A, *B*, *C*, *D*, and *E* are the designations given to the derived groups from both I and II. The four derived pairs of groups of isolation lines between which comparisons are made are groups IB, IIB; IC, IIC; ID, IID; IE, IIE. Group II was derived from Group IA by continuing, without endomixis, 24 of the lines of this group.

e stands for climax of endomixis. This symbol is put in on the day on which endomixities were isolated from the mass cultures.

T indicates the time of termination of the experiment.

Time in days, from the initial endomixis in Group I, is shown on the horizontal axis at the top of the diagram. The number of days between successive endomixes is shown at the bottom of the diagram.

endomixis and the resulting mortality in them was determined. In these groups (IA, IB, IC, ID, and IE, Fig. 1), therefore, the mortality after interendomixitic intervals of 31, 56, 81, 102, and 125 days, respectively, was ascertained. At the same time that each of these

groups (except the first) was being studied, there was examined a control group of lines that went through endomixis at the same time, but had instead an interendomictic interval of normal extent (21 to 25 days). The four control groups (II_B , II_C , II_D , and II_E) were obtained by inducing four successive endomixes at the proper time in descendants of the group I_A . Comparisons of mortality were thus made between the following pairs of groups: I_B (previous interval 56 days) with II_B (previous interval 25 days); I_C (previous interval 81 days) with II_C (interval 25 days); I_D (interval 102 days) with II_D (interval 21 days); and I_E (interval 125 days) with II_E (interval 23 days).

In this experiment, the animals employed were all descended vegetatively from one which was isolated from a Johns Hopkins stock

TABLE I

Number of fissions from climax of endomixis until death in sixty non-viable lines among one hundred and twenty exendomictic lines followed through until death or the next induction of endomixis.

(In these lines, the maximum interendomictic interval was 71 fissions.)

Number of Fissions until Death	Frequency
1.....	2
2.....	3
3.....	4
4.....	6
5.....	13
6.....	7
7.....	7
8.....	6
9.....	6
10.....	1
14.....	1
27.....	1
29.....	1
30.....	1
37.....	1

mass culture of Woodruff's long-lived Yale race. The organisms were cultivated throughout according to the methods described by Sonneborn (1936). Samples of all lines, except those not carried through till the next endomixis, were stained daily to determine the nuclear condition.

*Relationship of the Interendomictic Interval to the Percentage
of Mortality after Endomixis*

The percentage mortality was computed as follows: In each group, each endomictic animal was cultivated as a single daily isolation line until the fifteenth fission after the climax of endomixis. The per-

centage of lines that died during this period was the percentage mortality for the group. The period of 15 fissions was decided upon as a conservative standard in view of the facts that the process of endomixis itself lasts not more than 9 or 10 fissions and that experience of previous investigators (e.g. Caldwell, 1933) as well as our own showed that deaths rarely occur in the interval from the tenth generation after the climax of one endomixis until the following endomixis. Table I gives the frequency of death at various stages of the interendomictic interval in the material here investigated. As appears from the table, deaths occurred but rarely after the tenth fission.

The results of the experiment are summarized in Tables II to IV. Table II gives a general view of the relation of mortality after endomixis to the length of the previous interendomictic interval. As appears in the table, the percentage mortality increases as the previous interendomictic interval increases. It rose from 32.2 per cent after

TABLE II

Relation of mortality after endomixis to length of previous interendomictic interval.

Previous Interendomictic Interval in Days	Percentage of Mortality after Endomixis	Number of Endomictic Lines Observed
21.....	32.2.....	90
23.....	62.5.....	48
25.....	59.3.....	91
25.....	57.0.....	79
31.....	66.6.....	48
56.....	71.4.....	49
81.....	79.2.....	53
102.....	89.3.....	84
125.....	100.0.....	29

an interval of 21 days to 100 per cent after an interval of 125 days. The first five intervals, 21, 23, 25, 25, and 31 days are normal interendomictic intervals for Woodruff's stock of *Paramecium aurelia*. The last four intervals of 56, 81, 102, and 125 days are abnormally long intervals for this stock. It will be noted that even in normal interendomictic intervals the percentage of mortality was high.

It will be observed, from an examination of Table II, that there may be great differences in mortality after endomixis even when the intervals between endomixes are practically the same. Such a difference appears between the percentages of mortality after the 21- and 23-day intervals when the percentages of mortality were 32.2 per cent and 62.5 per cent, respectively. Obviously there are factors other than interendomictic interval involved in the production of mortality. Environmental factors probably play an important rôle here. Therefore, it

was essential, for the purposes of this study, to make simultaneous comparisons of groups kept under identical cultural conditions.

The results of such simultaneous comparisons are given in Table III. There the percentages of mortality from endomixis after four abnormally long interendomictic intervals are compared with the percentages of mortality from concurrent endomixes after four normal intervals. Thus, when the interendomictic interval was 56 days, there was 71.4 per cent mortality, as compared with 59.3 per cent in the concurrent group with a normal interval of 25 days. When the interval was increased to 81 days, the mortality was between 79.2 and 84.0 per cent (the exact figure depending upon how many of 20 animals that died without dividing—and hence without a determination of whether they had been in endomixis—were in endomixis), as compared with

TABLE III

Relation between length of previous interendomictic interval and percentage of mortality after endomixis.

Groups with Normal Interendomictic Intervals			Groups with Long Interendomictic Intervals		
Name of Group	Previous Interendomictic Interval in Days	Percentage Mortality after Endomixis	Percentage Mortality after Endomixis	Previous Interendomictic Interval in Days	Name of Group
II _B	25	59.3	71.4	56	I _B
II _C	25	57.0	79.2	81	I _C
II _D	21	32.2	89.3	102	I _D
II _E	23	62.5	100.0	125	I _E

57.0 per cent in the concurrent group with a normal interval of 25 days. When the interval was still greater, 102 days, the mortality likewise increased to 89.3 per cent as compared with 32.2 per cent in the controls with a normal interval of 21 days. Finally, when the interval reached 125 days, the mortality was 100 per cent, as compared with 62.5 per cent in the controls with a normal interval of 23 days. The effects of still greater intervals could not be studied because efforts to induce endomixis at intervals of 148, 158, and 161 days all failed; all but a few of the animals in the induction cultures died within 48 hours.

The results of the experiment, as summarized in Tables II and III, show clearly that as the time between two successive endomixes increases, the percentage of lines that are unable to survive the second endomixis also increases until eventually no animals can survive.

Relationship of the Interendemic Interval to the Number of Generations between Endomixis and Death

The relationship between the length of the previous interendemic interval and the number of generations which the non-viable exendemic lines lived after the climax of endomixis is shown by the data in Table IV. When the previous interval was of normal extent, i.e. about 25 days, as was the case in the control groups, the mean number of generations which the lines lived after the climax of endomixis remained fairly constant, ranging only from 5.0 to 5.9 generations.

TABLE IV

Length of life in number of generations from climax of endomixis until death, in the non-viable exendemics, in relation to prior interendemic interval. (The number dying without fission was not determined in experiments 1 and 2.)

Ex-periment	Group	Inter-endemic Interval in Days	Number of Generations from Climax of Endomixis until Death															Total Non-viable Exendemics	Mean	
			0	1	2	3	4	5	6	7	8	9	10	11	12	13	14			15
1	II _B	25	?	1	2	3	5	15	7	8	8	4	1					54	5.9	
	I _B	56	?	1	1	7	9	5	6	3	2			1				35	4.8	
2	II _C	25	?	1	1	5	6	13	7	4	2	1	1	2		1	1	45	5.8	
	I _C	81	?	7	3	3	6	9	8	4	1	1						42	4.4	
3	II _D	21	3	1	2	3	3	3	7	2	1	1	2	1				29	5.0	
	I _D	102	6	1	5	13	18	13	8	4	1	4	2					75	4.4	
4	II _E	23	4		3	4	3	5	1	2	2	1	1		1		2	1	30	5.5
	I _E	125	21	1	2	2	2					1						29	0.9	

Among the experimental groups the mean number of generations decreased from 4.8 generations when the interval was 56 days to 0.9 generation when the interval was 125 days.

In contrast to the steady increase in mortality rate with increasing interendemic intervals, the mean survival period shows no such steady change. When the interendemic interval was twice as great as normal the survival period decreased but little. Even when the interendemic interval was 3 or 4 times as great as normal, the survival period was decreased, on the average, by only about one fission.

Differences of this magnitude are of doubtful significance because the method of determining when the climax of endomixis occurred involved an uncertainty. No direct observation could be made on the endomictic animals removed from the induction cultures to begin the experimental and control groups; but the next day one or more products of their fission were stained and the nuclear condition recorded. On the basis of the number of fragments of the old macronucleus, their size and the intensity of stain, and the size, form, and intensity of stain of the new macronucleus or its anlage, an estimate could be made of how many fissions had occurred since the climax of endomixis; but such an estimate may often be in error by one or two fissions. For this reason, little significance is attached to the slight differences among the preceding groups in the mean number of generations that the non-viable lines survived.

In the group with the longest interendomictic interval, however, the difference is so great as to be unquestionably significant. After an interendomictic interval of 125 days, the non-viable exendomictics went through only one-sixth as many fissions as the non-viable exendomictics with normal prior interendomictic intervals. Indeed, 72.4 per cent of them failed to divide at all after the climax of endomixis. On the third day without fission they were all stained and found to contain fragments of the old macronucleus, but no new anlage or macronucleus. It might be suggested that about three-fourths of the parent lines had lost their micronuclei before endomixis was induced. In such animals only the destructive phases of endomixis can take place as there is no reserve micronucleus from which a new macronucleus can be formed. Loss of the micronucleus after long omission of endomixis has in fact been observed by others in this laboratory (unpublished).

Discussion

1. In view of the complex and superficially paradoxical relations between endomixis and mortality, it has sometimes been held that endomixis is neither a definite phenomenon nor a normal one, but that it is a pathological response of the organism to adverse conditions, the type of response and its consequences varying with the degree of unfavorableness of the environment. In the present work, effects due to differences of environment were avoided by systematically exchanging culture medium between the various groups compared and by restricting comparisons to groups examined at the same time under the same cultural conditions. Thus, the differences in mortality after endomixis were not consequences of environmental differences. Nor were they due to the cumulative action of unfavorable conditions, for in



each case the groups compared had been subjected equally long to the same conditions. The differences in mortality after endomixis must therefore have been due to the difference in the prior interendomictic interval. The fact that mortality is increased after longer intervals shows that the frequent occurrence of endomixis is an advantage to the stock. In view of this, the interpretation of endomixis as pathological is untenable. Similar conclusions were reached by Kimball (1937) from a study of the precise ratios in which sex segregates after endomixis in this species.

2. The view that Protozoa are potentially immortal and that natural death does not exist among them became popular during the last quarter of the nineteenth century. It has long been known, however, that while a race as a whole may be potentially immortal, certain members of the race are doomed, from internal causes, to die. Thus, Jennings (1913) and others showed that conjugation often resulted in unavoidable death. Erdmann and Woodruff (1916), Jennings, Raffel, Lynch, and Sonneborn (1932), Raffel (1932), Caldwell (1933), Sonneborn and Lynch (1937), and others have shown that endomixis likewise often results in death. Woodruff (1917), Sonneborn (1935), and Jennings and Sonneborn (1936) have shown that long omission of endomixis ultimately results in death. To these intrinsic causes of death in *Paramecium* the present paper adds another: The mortality at endomixis is directly proportional to the preceding interval without endomixis.

SUMMARY

1. Using an interendomictic interval of 20 to 30 days as a standard, it was shown that intervals approximately two, three, four, and five times this long resulted in progressive increases in mortality after endomixis until 100 per cent mortality occurred.

2. At intervals greater than this, the animals died before endomixis could be induced under conditions favorable for its induction.

3. The mean number of generations which non-viable lines survived the climax of endomixis was 5.0 to 5.9 generations when the previous interval was of normal extent. After an interval of 125 days, survival dropped greatly to a mean of 0.9 generation.

4. The results are shown to disagree with current interpretations of mortality at endomixis based on the concept of endomixis as a pathological process.

5. The results show that unusually long interendomictic intervals are, like other previously known conditions, a cause of "natural death" in Protozoa.

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